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OF PROTEINS FOR FOODS

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## FUNCTIONAL PROPERTIES OF PROTEINS FOR FOODS

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This thesis is based on the following communications, referred to in the text by their Roman numerals:

- I        A.-M. Hermansson, Functional Properties of Proteins for Foods - Solubility, AUL:s halvårsskrift, 2, (1973).
- II       A.-M. Hermansson, Functional Properties of Proteins for Foods - Swelling, Lebensm.-Wiss. u. Technol. 5(1) 24, 1972.
- III      A.-M. Hermansson, Functional Properties of Proteins for Foods - Flow Properties, submitted for publication.
- IV      A.-M. Hermansson & C. Åkesson, Functional Properties of Added Proteins Correlated with Properties of Meat systems.  
1. Effect of Concentration and Temperature on Water Binding Properties of Model Meat Systems, submitted for publication.
- V        A.-M. Hermansson & C. Åkesson, Functional Properties of Added Proteins Correlated with Properties of Meat Systems.  
2. Effect of Salt on Water Binding Properties of Model Meat Systems, submitted for publication.
- VI      A.-M. Hermansson, Functional Properties of Added Proteins Correlated with Properties of Meat Systems.  
3. Effect on Texture of a Meat Product, submitted for publication.





## BACKGROUND

The development of protein products for human consumption, such as protein concentrates, isolates and defatted meal is the subject of intensive research at various locations all over the world. Some current protein sources are blood, coconuts, cottonseed, eggs, fish, green plants, milk, peanuts, rapeseed, soybeans, single cells and sunflowerseed. Of greatest importance for the current market are protein products from soybeans, milk and eggs.

The market for protein products is increasing enormously. In a survey made by Hammonds and Call (1972) on the future market of protein products in the U.S., the maximum market potential was estimated to be 3.1 billion pounds yearly. The market in the U.S. in 1970 was approximately 0.9 to 1.1 billion pounds. Dairy products, baked foods, pet foods and processed meat stand out from other food products in terms of volume potential. These four alone were believed to account for 2.7 billion pounds, or 86% of the total potential market. The figures mentioned are based on pure protein. The canned and processed meat industry was identified as having the largest growth potential over the next 10 years. A technological forecasting probe of the meat industry predicted that extenders and analogs combined would reach 10% of all domestic meat consumption by 1985, and this level was felt to be virtually assured before the year 2000. According to Hammonds and Call this growth would carry proteins used in the meat industry from the present level of 92 million pounds (145 million pounds of protein products) to 1.56 billion pounds (2.45 billion pounds of protein products) in 15 years.

This forecast is made for the U.S. market, and although the figures can only be considered approximate, they give an idea of the enormous expansion of the protein market. The market in Sweden is naturally of another dimension. As an example, about 1200 tons of soy protein products were used in Sweden 1972 compared to 72000 tons in the U.S. in 1970. In both markets the annual growth is at present about 19%.

The success of a new protein product is determined by its price and functionality. Thus, apart from having intrinsically satisfactory properties such as nutritional value, flavour and colour, the protein





products should possess certain functional properties, which make them compatible with the various foods to which they are to be added. Consequently, a very important aspect of the development of processes for the production of new protein ingredients is to establish their functional physico-chemical properties, which can be defined as those giving information about how the protein will behave in a food system.

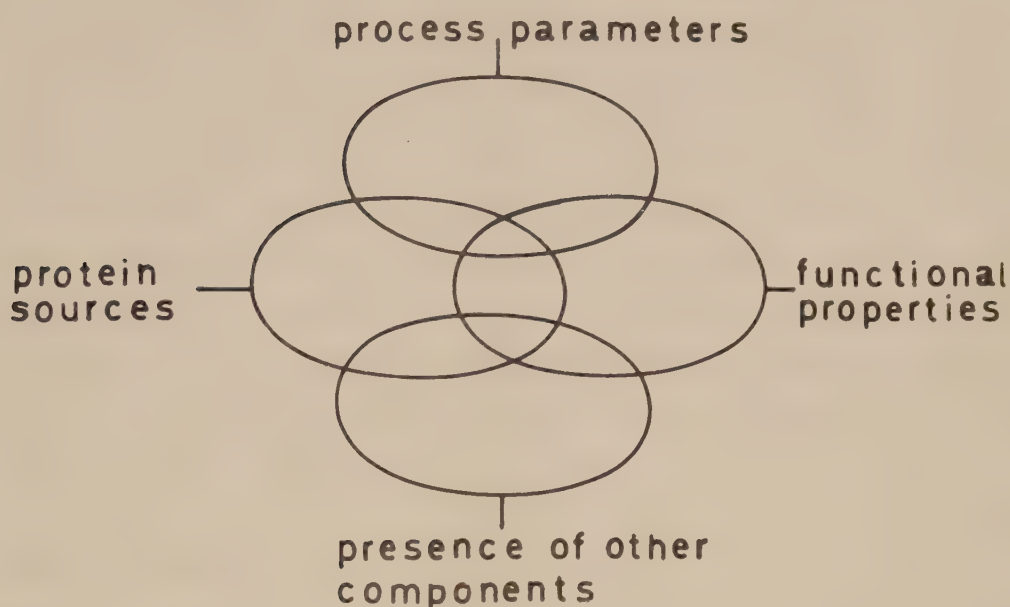
The problem of functional characterization can be approached in two different ways. The traditional method is to test a new protein product in a wide range of different types of foods. This trial-and-error technique is both time consuming and expensive and gives very limited information about the behaviour of the added protein. An alternative approach is to study simple model systems with regard to functional properties from which information about the complex nature of the proteins can be obtained. If then the functional properties have been shown to correlate with properties of food systems, it will only be necessary to test the protein in a restricted number of food formulas. Although much data has been published on studies of different functional properties, very little has been published on how properties of the protein products can be correlated with properties of food products to which proteins have been added. Hitherto only Yasumatsu et al. (1972) have published correlations of some properties of soy proteins with properties of doughs and fish paste products by means of an ordinary factor analysis procedure.

Functional properties of interest are solubility, swelling, viscosity, water-holding capacity, emulsion, foam and gel properties. This thesis includes studies on solubility, swelling, viscosity, gel strength and water-binding properties, but some studies have also been made on other functional properties (Hermansson et al. 1971, Hermansson et al. 1974). Functional properties are highly dependent on the conditions of the production processes, both for the finished food products and for new protein products. Parameters of importance are pH, protein concentration, salt concentration, temperature, nature of organic solvent, drying conditions, energy of mixing processes, etc. Knowledge of functional properties is therefore of importance not only for the choice of application for a new protein product but also for the optimization of new processes. The complex dependence of functional properties on various factors is qualitatively illustrated by the Venn-diagram shown in figure 1.





Figure 1. A Venn-diagram illustrating the complex dependence of functional properties on various factors.



#### THE AIM AND DESIGN OF THE PROJECT

The aim of this study was to make a systematic investigation of functional properties dependent on water-protein interactions with respect to parameters such as protein concentration, salt concentration, pH and temperature. The effect of the functional properties on model meat systems and on a finished food product was then to be estimated. The object of the project was rather to give general relations between functional properties and their correlations with food properties than to give detailed interpretations of the results obtained.

The design of the project is schematically illustrated in appendix 1.

#### Functional properties

Functional properties can be classified into those depending on water-protein, water-protein-fat and water-protein-air interactions. It seemed natural to start with those depending on water-protein interactions since they have a fundamental bearing on most biophysical parameters. Properties studied were solubility, swelling, gel strength and flow properties.





Much work was concentrated on the choice of methods. As functional properties are to be tools both for the choice of application and for process optimization, accurate knowledge about the methods and the effect of experimental conditions is important. Many published results are unfortunately based on poorly developed methods. Briskey (1970) was one of the first to stress the need of improved methodology for quantitating protein functions. Furthermore, methods for functional characterization should be simple and inexpensive and the methods developed can be used in any normally equipped laboratory without high investments. Of importance is also that the methods could be developed into standard methods for many different protein systems. Therefore three completely different protein systems were chosen for this study. They were a soy protein isolate (Promine-D), a caseinate (Sodinol) and a whey protein concentrate.

#### Protein products

Protein products for human consumption are very complex systems, in which the protein composition and molecular structure of protein components are very little known. Of the available protein sources, soybeans and milk are two of the most investigated. Soy protein, caseinate and whey protein were chosen for this study both because they are completely different as mentioned above and because the functional characteristics of these protein systems in some cases can be rationalized from available information on their molecular structure and its dependence on environmental conditions.

The major soy proteins, the 7S and 11S globulins, are characterized by a complex quaternary structure and molecular weights of ca 200 000 and 350 000, respectively. They are known for their sensitivity to the ionic environment, easily undergoing association-dissociation reactions with changes in ionic strength. The 7S fraction makes up about one third of the total soy proteins and the 7S globulin represents more than one half of this fraction. The 11S fraction makes up another one third and the 11S globulin accounts for most of this fraction (Wolf 1970, Koshiyama 1968).





The protein components of casein as well as those of soybean make up a very complex protein system, the structure of which is not fully known in spite of intensive research. Casein molecules are polymerized to structures usually referred to as micelles. The molecular weight distribution of these micelles can be very broad, and molecular weights from  $10^5$  -  $10^8$  have been observed. The micelles have a composition of ca 55%  $\alpha$ -casein, 30%  $\beta$ -casein and 15%  $\kappa$ -casein.  $\alpha$ - and  $\beta$ -casein have both a high degree of hydrophobicity. The micelle-like structures are stabilized by calcium-phosphate bonds.

In contrast to the proteins of soybean and casein, the whey proteins do not form high molecular complexes. The  $\beta$ -lactoglobulins making up 59% of the whey proteins have a molecular weight of 18000. They are known to exist as dimers at pH 5.2 and to dissociate to monomers on either side of this pH. The  $\alpha$ -lactalbumins making up to 22% of the whey proteins have a molecular weight of 16000 and are known to associate to dimers and trimers at low pH (McKenzie 1971).

Some additional studies, not discussed in this thesis, have also been made on protein products produced in Sweden such as rapeseed, leaf and fish protein concentrate. The effect of different solubilization procedures on the functional properties was studied in the case of rapeseed and fish protein concentrate (Hermansson et al. 1971; Hermansson et al. 1974).

### Food systems

The canned and processed meat industry is expected to have the highest potential for protein products. Meat was therefore chosen as the food system for these investigations. Meat products are, however, very complex and difficult to control. Well-defined model meat systems prepared under controlled conditions were therefore used for the correlation studies. The investigation on model food systems concerned correlation of the studied functional properties depending on water-protein relations with the water-binding properties of the meat systems. Lack of water-binding properties will result not only in weight losses, but will also influence texture (Hamm 1972; Galloway et al. 1973).



For a finished food product texture is one of the most important properties. As texture and water-binding are related, it was of interest to see whether any of the relationships established in the model meat systems between water-binding properties and functional properties of the proteins added had any validity for the texture of a real meat product.

## SUMMARY OF THE RESULTS

### Functional properties

#### Solubility (I)

In the extraction and precipitation of proteins during production, information about changes in solubility is of prime importance. The importance of solubility for food products such as beverages is obvious. For semi-solid food products, data on solubility in combination with data on other properties give valuable indications on the functionality of a new protein product.

The solubility concept used for proteins is dependent on experimental conditions and cannot be defined in terms of solubility products used for salts etc. The method involves moderate shaking at 25°C of 1% dispersions for 45 min, centrifugation at 40000 x g for 30 min, and determination of the nitrogen content in the supernatant by a standard Kjeldahl procedure.

The measurements showed that complex protein systems have quite different solubility profiles with respect to variables such as NaCl concentration, pH and temperature. This is due to the composition of the protein product, complexity in molecular structure and conditions of the production processes. The solubility depends on the balance between attractive and repulsive forces. With variation of pH the net charge is changed and thereby the electrostatic balance. At the isoelectric point (IP) the net charge is zero and the solubility normally shows a minimum. If proteins with different IP:s are present in a protein system, a solubility profile with several minima and inflection points can be obtained. Salts may influence the solubility very differently. Salts can act by direct binding to the protein, by indirect action through effects on the structure and properties of the solvent, or by a combination of these effects





(Hippel and Schleich 1969).

Solubility profiles may be very complex and difficult to interpret. Lack of mobile counter ions when measurements are made in dist.  $H_2O$  or at very low ionic strengths might result in high intramolecular repulsive forces at pH:s outside the isoelectric region. The degree of denaturation caused by processing also influences the solubility profile. By denaturation intermolecular bonds may form which cannot so easily be overcome by electrostatic repulsive forces caused by pH changes. Solubility as such should not be used as a criterion for denaturation, but when a protein system shows a low solubility within a broad pH range this is a sign of severe denaturation. Typical solubility profiles for denatured proteins were obtained for a rapeseed protein concentrate and a fish protein concentrate (I, figure 7).

Of the three test proteins, soy protein is very sensitive to changes in the ionic environment. The solubility of both Promine-D and a "native" soy protein isolate was markedly influenced by changes in NaCl concentration under various conditions (I, figures 2 and 3). At pH around 7 the solubility decreased with increasing NaCl concentration to  $I = 0.1$ , whereupon it slowly increased to  $I = 1.0$  ( $I =$  ionic strength). The observed solubility changes were in accordance with reported association-dissociation reactions. Association was shown to occur at  $I = 0.1$ , and at very low ionic strengths, the quaternary structure was shown to be unstable and to dissociate. The dissociation can be caused by strong intramolecular repulsive forces in the absence of mobile counter ions.

The other complex protein system, caseinate, was characterized by very drastic solubility changes. At pH:s around 5, for example, the solubility increased from 10% to 90% within a half pH unit (I, figure 6). In the range 0 to 1.0 M NaCl the solubility of caseinate was little affected, in contrast to the solubility of Promine-D, but caseinate was drastically salted out at concentrations of about 2 M NaCl (I, figure 3).





The solubility of the less complex WPC system did not show such as strong pH and NaCl concentration dependence as soy protein isolate and caseinate. One reason for this may be that the smaller whey proteins are difficult to sediment even in the absence of repulsive forces under the experimental conditions. The solubility in the isoelectric region was for example as high as 63% (I, figure 6).

### Gelation (I, II, V)

When protein dispersions at sufficiently high concentrations are heated, a gel may form. Gels are weak solids formed from a continuous network of macromolecules with an interstitial filling of a liquid. The unique property of gels is that they possess the main characteristics of a solid although their water content may be as high as 99%. The gel structure is of extreme biological importance. In foods the gel structure acts as a matrix holding not only water but also lipids carbohydrates and other species. Meat products, bread, cheese and gelatin can all be considered as protein gels. Denatured gels can, however, only form under highly specific conditions with just the right balance of attractive and repulsive forces. According to Ferry (1948), gelation is a two stage process: native protein → denatured protein (long strands) → associated network. The slower the second step, the higher the concentration of free denatured protein and the finer the network.

The gel strength of 10% protein dispersions in distilled water and in 0.2 M NaCl was studied. A qualitative method previously described by Circle *et al.* (1964) was used, in which the resistance of the gel to penetration by a T-shaped spindle in a Brookfield viscometer was measured. The spindle moved down in a helical path through the material, always measuring undisturbed material. The gels were considered as strong when the Brookfield "poise" exceeded 1000.

Gels were formed for Promine-D and WPC but not for caseinate due to the unique heat stability of the casein "micelles".

Promine-D formed very strong gels in distilled water. The gels formed were very smooth, and it was impossible to press out water. Strong gels formed at 65°C and the gel strength increased to 80°C, whereupon it rapidly decreased due to chemical breakdown of the structure



(II, figure 9). If salt was added, however, the gelation ability was markedly reduced and strong gels did not form until the temperature was raised to 100°C (IV, table 3).

For WPC the relations were inverted with respect to the effect of NaCl (V, table 1). In distilled water, WPC had poor gelation ability and strong gels did not form until the temperature was raised to 90°C (II, figure 9). By addition of salt, strong gels were formed at temperatures as low as 75°C. The gels of WPC were of another character than those of Promine-D. The gel structure was coarse and water was easily pressed out.

The different effects of salt on the gelation ability of the two protein products might be due to the different repulsive balances in the two cases. For soy proteins, with their complex quaternary structure, the presence of salt counteracts dissociation into subunits and thereby unfolding and step 1 in the gelation process (Wolf 1970; Koshiyama 1968, 1971; Catsimpoolas and Meyer 1970). For the whey proteins without complex quaternary structure the presence of salt favours protein-protein interactions and step 2 in the gelation process. The effect of salt on whey protein interactions as a function of temperature was also illustrated by solubility measurements of 1% dispersions (I, figures 8 and 9).

The addition of salt thus favoured protein-protein interactions both for soy and whey proteins, although salt had the opposite effect on gelation due to differences in molecular structure.

The difference in gel structure between Promine-D and WPC gels should be of importance for the function of these proteins in heat-treated food products where good water-binding properties are required.

### Swelling (II)

Swelling, when defined as the spontaneous uptake of water, is the first step in the solvation process. If swelling is unlimited the proteins will solvate, otherwise swelling will proceed until it is limited by various intermolecular forces in the swollen sample.

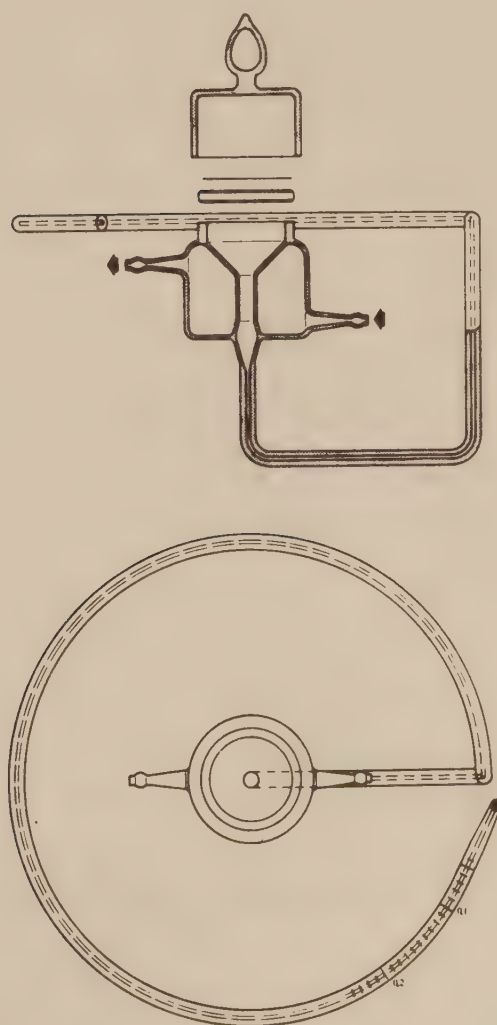




As most protein products of interest as food ingredients are neither completely soluble nor insoluble in water, the swelling cannot be defined rigorously, and any description of their swelling behaviour must be empirical. In a typical semi-solid food product, a protein additive is supposed to bind a certain limited amount of water. This implies that for protein products swelling should be measured in terms of the ability to take up water in amounts smaller than those producing low viscosity dispersions.

A method was developed for the study of the relative swelling of proteins in an apparatus originally designed by Baumann (1967) for sorption studies on porous inorganic solids. The design of the apparatus is shown in figure 2.

Figure 2. The swelling apparatus.





The glass equipment consists of a thermostated funnel connected to a horizontally located capillary. A sample is dusted on to a wetted filter paper placed on top of the funnel, which is filled with liquid. The uptake of fluid can then be followed in the capillary.

The swelling characteristics of Promine-D, caseinate and WPC were of entirely different characters (II, figures 1, 3, 4, 5). The swelling of Promine-D could be regarded as limited and the swelling of caseinate and WPC as unlimited.

Promine-D showed very good swelling ability in distilled water, which was markedly reduced by the addition of salt (II, figure 7). Caseinate showed a high degree of swelling before solvation. WPC finally had a poor swelling ability. Similar to the solubility the swelling increased with pH on the alkaline side of the isoelectric point.

When protein gels, formed by heat treatment, were freeze-dried and ground, it was found that the protein network formation had increased the swelling ability. For Promine-D the swelling ability increased enormously in distilled water, and a very good correlation was found between swelling ability and gel strength before freeze-drying (II, figures 9 and 11).

Thus, the two complex protein products Promine-D and caseinate both showed a high initial water uptake, although their swelling was entirely different in character. The smaller globular whey protein possessed poor swelling ability. A high swelling ability could, however, be induced by the formation of a protein network.

### Flow properties (III)

Flow properties of protein dispersions are of considerable interest for the production of protein products, both for the design of processes, and for functional characterization.

Flow properties can often be expressed by the power equation

$$\tau - \tau_0 = k \cdot D^n$$





where  $\tau$  = shear stress ( $\text{dyn/cm}^2$ );  $\tau_0$  = yield value ( $\text{dyn/cm}^2$ );  
 $D$  = shear rate ( $\text{s}^{-1}$ ).  $n$  is referred to as the flow behaviour index;  
 for Newtonian fluids  $n = 1$ , and any other value indicates deviation  
 from Newtonian flow.  $k$  is the parameter corresponding to viscosity,  
 but only when  $n = 1$ , does  $k$  have the dimensions of viscosity.  $k$  is  
 often referred to as the consistency index. The viscosity term ( $\eta$ )  
 can be obtained by differentiation of the power equation

$$\eta = \frac{\delta\tau}{\delta D} = k \cdot n \cdot D^{n-1}$$

The flow properties of 4 - 20% protein dispersions were studied  
 as a function of pH and salt concentration. The measurements were  
 carried out in a Haake Rotovisko model RVI at 25°C. Apart from the  
 soy protein isolate Promine-D and the caseinate Sodinol, some other  
 commercial protein products, as well as a "native" soy protein  
 isolate, were tested.

The results showed that the flow properties of protein dispersions  
 differed greatly due to origin and processing conditions. Some  
 understanding of the flow characteristics can be obtained from  
 consideration of differences in the quaternary structures of the  
 three protein systems studied, the degree of denaturation due to  
 processing, and other functional properties such as swelling and  
 solubility.

The flow properties of Promine-D were characterized by low  $n$ -values  
 and the existence of yield values at concentrations above 8% in  
 distilled water at pH 7 (III, table 1). The flow characteristics  
 of Promine-D in distilled water strongly indicate the presence of  
 protein-protein interactions leading to the formation of a protein  
 network at high concentrations. The "native" soy protein isolate,  
 on the other hand, showed no yield values, higher  $n$ -values and lower  
 $k$ -values. Furthermore, the native soy protein had a lower swelling  
 ability and a higher solubility (III, table 4). Processing was  
 considered to have caused the formation of swelling aggregates  
 responsible for the flow characteristics of Promine-D. The addition  
 of salt had a viscosity-decreasing effect on all soy protein isolates  
 studied. Furthermore, yield values disappeared or were strongly  
 reduced (III, table 2). As not only viscosity but also swelling



and solubility were strongly reduced by the addition of salt, the change in flow properties can be understood from the presence of less swelled, more rigid aggregates and less solvated protein molecules in the continuous phase. The same relations among flow properties were obtained with increasing pH, where the increase in net negative charge caused increased solubility, swelling, viscosity and yield value.

The flow properties of caseinate dispersions were characterized by high  $n$ -values and the absence of yield values, with respect to changes in protein concentration, pH and salt concentration (III, tables 1, 2, 3 and 4). The viscosity parameters increased enormously with concentration. The high  $n$ -values close to one and the absence of yield values indicated that the increase in viscosity was more likely to be due to protein-water interactions (hydration) than to strong protein-protein interaction forces. It was suggested that the amount of swollen not fully solvated caseinate particles in the dispersions were responsible for the enormous increase in viscosity with concentration. At high concentrations, the particles will come closer together, causing friction and weak interaction forces between the particles. In contrast to the salt effect of soy protein dispersions, the addition of salt caused an increase in the viscosity parameters of caseinate dispersions. The viscosity dependence on pH seemed to be more complex for caseinate than for the other protein systems and a maximum in viscosity was reached at pH 9.1 - 10.0, whereupon it rapidly decreased. Similar maxima for caseinate dispersions have been observed by Salzberg and Georgevits (1956), Hayes and Muller (1961) and Puri et al. (1972).

Whey protein dispersions had low viscosity over a broad concentration range, with high  $n$ -values and no yield values. At high concentrations, (16 - 20%)  $n$ -values decreased, and at 18% and 20%, yield values developed (III, table 1). Factors such as pH and ionic strength had little effect on viscosity, as well as on swelling and solubility properties of WPC (III, tables 2 and 3). This does not mean that these variables have no influence on the molecular properties, such as net charge and association equilibria, but that the induced changes of the smaller whey proteins were too small to be reflected by measurements of the above-mentioned functional properties.





### Correlations among functional properties (V. VI, II, III)

The results have shown that the functional properties are not independent. Swelling is the first step in the solvation process, and swelling and viscosity are highly intercorrelated. Solubility, swelling and viscosity measured at 25°C cannot be used to predict gelation, but when a protein network forms by heat treatment, solubility is decreased and swelling ability is increased.

The differences in the complex structures of the test proteins influenced all the properties studied. The solubility as such gave no information on complexity, and both caseinate and WPC were highly soluble at pH 7. The solubility profiles as a function of variables such as pH and salt concentration, however, indicated complexity as well as denaturation effects. The two complex systems Promine-D and caseinate both possessed high swelling ability and viscosity parameters, although the nature of the swelling and flow were entirely different in character. The difference can probably be understood in terms of the stronger protein-protein interactions of soy protein than of caseinate. The unique stability of the casein micelles to heat made gelation of caseinate impossible. Caseinate can, however, form gels at pH > 12.5 (Plomly et al. 1951).

The relationships among the functional properties can be expressed qualitatively by the Venn-diagram shown in figure 3, or quantitatively by the clustering tree shown in figure 4.



Figure 3. Relationships among functional properties illustrated by a Venn-diagram.

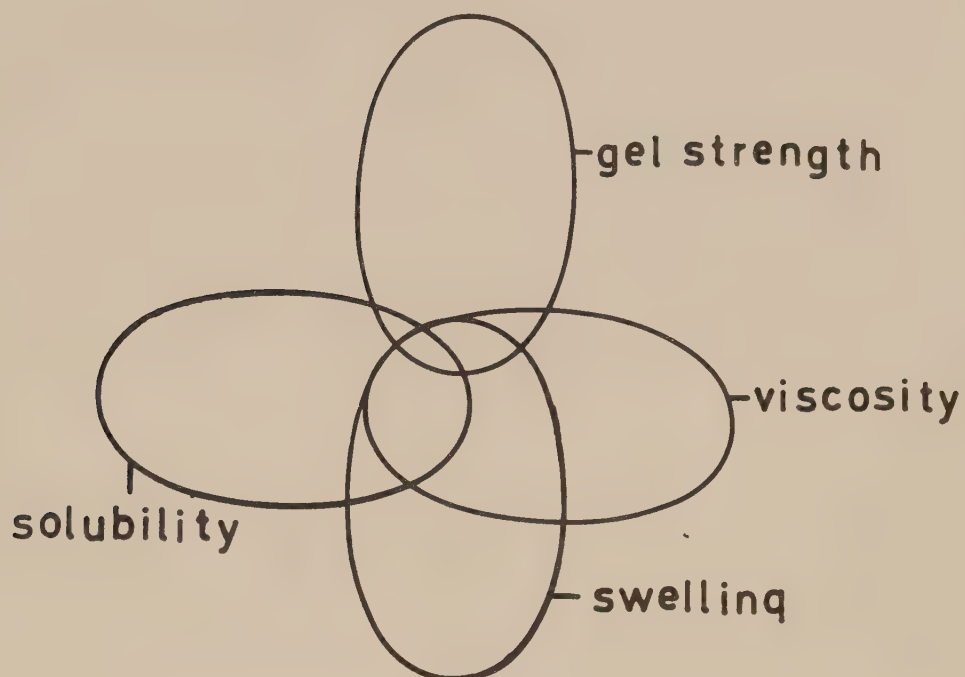






Figure 4. Relationships among functional properties calculated from hierarchical cluster analysis.

Functional properties are: 1 = solubility at 25°C, 2 = solubility at 25°C after heat treatment at 80°C, 3 = swelling at 20°C, 4 = swelling at 20°C after heat treatment at 80°C, 5 = viscosity of 10% dispersions measured at 42 s<sup>-1</sup>, 6 = viscosity of 10% dispersions measured at 1142 s<sup>-1</sup>, 7 = viscosity of 12% dispersions measured at 42 s<sup>-1</sup>, 8 = viscosity of 12% dispersions measured at 1142 s<sup>-1</sup>, 9 = viscosity of 10% dispersions measured at 15 s<sup>-1</sup> after heat treatment at 80°C. All viscosity measurements were made at 25°C. 10,11 = Brookfield data at 10 and 12% protein gels formed by treatment at 80°C.

| a        | N     |     |     |       |       |       |       |   |   |    |    |
|----------|-------|-----|-----|-------|-------|-------|-------|---|---|----|----|
|          | 0     |     |     |       |       |       |       |   |   |    |    |
| AMALG.   | 1     | 2   | 5   | 6     | 4     | 3     | 8     | 7 | 9 | 10 | 11 |
| DISTANCE |       |     |     |       |       |       |       |   |   |    |    |
|          | *     | *   | *   | *     | *     | *     | *     | * | * | *  | *  |
|          | I     | I   | I   | I     | I     | I     | I     | I | I | I  | I  |
| 0.012    | I     | I   | I   | I     | -+-   | I     | I     | I | I | I  | I  |
|          | I     | I   | I   | I     | I     | I     | I     | I | I | I  | I  |
| 0.142    | -+-   | I   | I   | I     | I     | I     | I     | I | I | I  | I  |
|          | I     | I   | I   | I     | I     | I     | I     | I | I | I  | I  |
| 0.161    | I     | -+- | I   | I     | I     | I     | I     | I | I | I  | I  |
|          | I     | I   | I   | I     | I     | I     | I     | I | I | I  | I  |
| 0.228    | I     |     | --+ | --    | I     | I     | I     | I | I | I  | I  |
|          | I     |     | I   |       | I     | I     | I     | I | I | I  | I  |
| 0.496    | ----- | +   | --  |       | I     | I     | I     | I | I | I  | I  |
|          |       | I   |     |       | I     | I     | I     | I | I | I  | I  |
| 1.556    |       | I   |     |       | -+-   | I     | I     | I | I | I  | I  |
|          |       | I   |     |       | I     | I     | I     | I | I | I  | I  |
| 3.371    |       |     | --+ | ----- |       | I     | I     | I | I | I  | I  |
|          |       |     | I   |       |       | I     | I     | I | I | I  | I  |
| 5.016    |       |     |     | -+    | ----- |       | I     | I | I | I  | I  |
|          |       |     |     | I     |       |       | I     | I | I | I  | I  |
| 5.896    |       |     |     |       | -+    | ----- |       | I | I | I  | I  |
|          |       |     |     |       | I     |       |       | I | I | I  | I  |
| 10.883   |       |     |     |       |       | -+    | ----- |   | I | I  | I  |



For the statistical clustering analysis, functional data measured at neutral pH in distilled water, and at various salt concentrations, were used. The parameters in the clustering tree are ordered with respect to degree of similarity, e.g. solubility parameter 1 is most similar to solubility parameter 2 and least similar to the gel strength parameter 11. The branching points illustrate statistical subgroups, and the smaller the statistical distance values of the branching points the more closely related are the properties. Viscosity parameters 5 and 6 are closely related to swelling parameters 3 and 4, and the swelling parameters are placed in between the four viscosity parameters. The branching point distance for solubility (1,2), viscosity (5,6) and swelling (3,4) is  $< 0.5$ , which is a sign of a relationship among these properties. As expected, the gel strength parameters 9, 10 and 11 formed a special group placed to the right in the clustering tree with high distance values of branching points. The results from the statistical analysis were in agreement with the previously observed relationships among the functional properties.

#### Model food systems (IV, V)

The aim of the study on model meat systems was to correlate the functional properties studied with water-binding properties of the meat systems. To focus all attention on the water-protein relations, model systems were used in which all visible fat was removed from the meat before it was ground and nothing but protein and water were added.

Two meat systems were used to avoid conclusions from a special case. Meat qualities common in minced meat products were used. They were beef brisket and pig shoulder. After removal of visible fat the fat content was 3.3% for the beef brisket and 2.8% for the pig shoulder system. To get equal samples the meat was mixed thoroughly, after which portions of ca 100 g were packed in casings of PVDC film and quickly frozen. The model systems were designed as follows: The quotient protein/water was kept constant at 0.2. When proteins were added, the calculations were based on percentage exchanged protein. This means that 30% and 50% exchanged protein are equal to 5.0% and 8.3% added protein of the total mixture.



Methods were developed to follow moisture loss of raw and heat treated meat systems. For the raw meat system a technique was used in which 20 g of meat mixture was centrifuged at 18000 rpm (40000 x g) for 30 min and the volume of released juice was measured. The method is referred to as  $ML_c$  (moisture loss after centrifugation).  $ML_c$  was calculated as  $(\text{ml released juice}/20) \times 100$ . For the heat-treated meat systems, a system referred to as  $ML_{ht}$  was used (ht = heated tube). 2 g of meat mixture was heated in small glass tubes for 30 min, the meat plug was then transferred to a net, weighed, centrifuged at 2000 rpm for 5 min and reweighed,  $ML_{ht}$  was calculated as  $(\text{weight difference}/\text{weight before centrifugation}) \times 100$ . Apart from moisture loss measurements, some additional test were made on the texture by a penetrometer method.

From the statistical point of view, the problem of this study consisted in mapping several independent variables (functional properties) into a set of dependent variables (water-binding properties). For this problem an approach of trying some appropriate multiple regression procedures seemed reasonable. Several types of regression models were tested, and it was found that the results obtained from a simple linear additive model turned out to satisfy the preset conditions. The selection of regression variables (functional properties) to be included in the statistical model was carried out by means of a computational procedure involving the calculation of regression equations with all possible combinations of variables. (With 11 different variables there are  $2^{11}$  possible outcomes to be ordered). Some of the reasons for using such a "complete enumeration technique" were a) complicated interactions of the potential variables were possible, which might confuse and contradict the outcomes from a step-wise elimination procedure; b) it could be assumed that there existed a number of equivalent or almost equivalent solutions for the different cases under consideration. The statistical calculations were made by C. Åkesson.

#### Without salt (IV)

Effects of protein incorporation were evident and alike in the beef and pig meat systems.





In the raw meat systems, when  $ML_c$  was studied as a function of concentration, exchange of meat by Promine-D resulted in the lowest moisture loss, which did not differ much from that of pure meat. Increasing levels of caseinate and WPC caused a pronounced increase in  $ML_c$  and was somewhat greater for WPC (IV, figures 3 and 4). Very good correlations between differences in functional properties and differences in moisture loss data were obtained (IV, table 5). Of the functional properties, solubility was positively correlated with moisture loss and the other functional properties negatively correlated with moisture loss. This observation was valid for all the statistical solutions obtained. The best statistical solution from the correlation of  $ML_c$  with functional properties had the very high correlation coefficient of 0.99. In this solution solubility explained 79%, swelling 10% and viscosity 10% of the variance. Of the tested proteins, the solubility of Promine-D was considerably lower than that of caseinate or WPC. Solubility, as well as  $ML_c$ , was high both for caseinate and WPC. The differences in  $ML_c$  between caseinate and WPC can be explained by the differences in their viscosity and swelling.

When  $ML_{ht}$  was studied as a function of heating temperature on the 30% and 50% level of exchanged protein, it was found that heating at 80°C gave a lower moisture loss when proteins were incorporated than heating at 70°C (IV, figure 5). In contrast to the raw meat systems, caseinate showed a higher moisture loss than WPC after heat treatment.

The results illustrate the importance of gelation ability. Both Promine-D and WPC can form gel structures when heated to 80°C, and thereby reduce moisture loss (IV, table 3). In meat systems, the added proteins might form gel structures within themselves or interact with the protein network.

When  $ML_{ht}$  was correlated with the functional properties of the added proteins, the correlation coefficients were as high as 0.97 - 0.98 in three out of five statistical solutions on the 50% level (IV, table 7). As expected, the gel strength parameters gave high contributions to the variance, and in the best statistical solution a gel strength parameter alone accounted for 94% of the variance. The same functional parameters as on the 50% level were involved on the 30% level



(5.0% of the total mixture), and the best solution on the lower level gave a correlation coefficient of 0.83.

#### With various amounts of salt (V)

Addition of salt (1% - 4%) is known to increase the water-binding properties of meat systems due to chloride binding and increased repulsive forces (Hamm 1972). As discussed previously salt affected the protein systems very differently. The gel strength of WPC was increased by the addition of salt, whereas the gel strength of Promine-D decreased. The viscosity of Promine-D decreased by the addition of salt, whereas the viscosity of caseinate increased, etc. A meat system in which part of the meat protein is exchanged by one of the protein systems can therefore be expected to show complex behaviour with respect to water-binding properties.

The addition of salt had an enormous decreasing effect on moisture loss in all the raw meat systems (V, figures 3 and 4). Most striking was the drastic decrease in moisture loss for the caseinate-meat system. The statistical solutions for raw meat systems gave rather high correlations, but as expected not as high as without salt (V, table 3). The best solutions had correlation coefficients of 0.80, and viscosity contributed the largest part of the variance. The increase in viscosity for caseinate and the corresponding drastic decrease in moisture loss of caseinate-meat systems was probably responsible for the high correlation of viscosity. In contrast to the systems without salt, neither solubility nor swelling gave high correlations in any of the solutions due to the fact that for caseinate neither of these properties was significantly affected by salt.

The influence of exchanged proteins on heat-treated meat systems containing various amounts of salt differed from their influence on the raw meat systems. The drastic effect of caseinate disappeared on heat treatment, and the caseinate-meat systems showed the highest moisture loss. The order was the same as in the absence of salt, with Promine-D addition giving the lowest moisture loss on the 50% level at all salt concentrations studied (V, figures 5 and 6). After heat treatment, very high statistical solutions were obtained on the 50% level. The best statistical solutions had correlation coefficients of 0.97 - 0.98 (V, tables 4 and 5). In the presence of salt, the solubility parameters accounted for most of the variance, but





significant contributions were given by viscosity and gel strength parameters. The poor correlations of the gel strength parameters in the presence of salt was due to the enormous increase in gel strength with salt concentration for WPC, which was not reflected in the moisture loss changes. As mentioned above, the WPC gels were coarser than those of Promine-D, and water was easily pressed out. The gel strength method used did not reflect this difference, and another way of characterizing the gels would probably give a higher correlation. The fact that WPC-meat systems showed lower moisture loss than caseinate only after heat treatment is, however, a strong indication of the importance of the gelation ability.

Correlations made on the 30% level for pig shoulder systems gave solutions with exactly the same functional parameters as on the 50% level, but the correlation coefficients were somewhat lower (0.87 for the best solution, V table 5). The moisture loss changes on this level differed very little from those of the pure meat systems (V, figure 7).

The results have shown that statistical regression methods could be used successfully to manifest the expected relationships between functional properties and properties of model food systems. In this study a simple linear additive regression model was used. In industrial applications for the control and optimization of real food systems, more complicated statistical models might have to be used. No statistical solution can, however, be explained on a physico-chemical basis without knowledge of the systems tested and the parameters involved. Nor can statistical methods as such be used to establish new hypotheses from relationships between unknown parameters. All statistical solutions for the systems containing various amounts of salt are, for example, not generally valid, due to the very specific salt-induced changes of the gel strength parameter for WPC.

#### A real food product (VI)

Several studies have been made on the relations between texture and water-binding properties (Hamm 1972; Galloway et al. 1973; Bauton et al. 1971). As moisture loss and texture changes were



inter-correlated it was of interest to see whether any of the fixed regression equations estimated on moisture loss changes with functional properties as independent variables had any validity for the texture changes of meatballs to which 4% proteins had been added.

A commercial meatball recipe was used for this study, with 10% of the total mixture replaced by 4% protein and 6% water. It was composed of 50% meat (pig and beef), potatoes, golden bread crumbs, dried milk, spices and water. Untreated Promine-D, caseinate and WPC and some freeze-dried and ground samples of Promine-D, and WPC gels were added. The freeze-dried and ground gels were studied in order to see how induced changes in functional properties of a protein product would affect properties of a finished food product.

The texture changes were measured instrumentally by an Instron Universal Testing machine and sensory evaluation for firmness by a panel of 10 experienced judges. The meatballs were served warm to the panel for sensory evaluation and cooled before instrumental tests were made. Although only 4% protein was added, significant changes were obtained, as measured both by instrumental and sensory evaluation (VI, table 3). The relative differences between the controls and products containing untreated proteins were in accordance with the previously observed differences in  $ML_{ht}$ , with Promine-D having the highest and caseinate the lowest values, both in an extrusion test and in the sensory evaluation. The effect of heat treatment differed between Promine-D and WPC. For Promine-D the changes in texture were in accordance with the differences in functional properties. For WPC, on the other hand, preheat treatment had a negative effect on the texture, which was also observed in moisture loss changes of the model system (VI, table 2). A reason for this negative effect of WPC could be that the soluble proteins of untreated WPC interacted with the meat proteins to form a gel structure when heated, and thus improved the water-binding properties of the meat systems. By preheat treatment the interaction possibilities of WPC were partly lost due to denaturation and reduced solubility. In contrast to Promine-D, the induced protein network of WPC had a poor swelling ability and could make no contribution to the water-binding properties of meat systems.



In the statistical analysis a number of fixed regression equations, estimated from moisture loss differences, were chosen. Although the correlation coefficients may be somewhat overestimated due to the limited amount of data, some very high correlations were obtained from the extrusion data (VI, table 4). The best equation gave a correlation coefficient of 0.88, when predicted texture differences calculated from the regression equation were correlated with the observed texture differences. For reasons discussed above, only pretreatment of Promine-D gave positive contributions to the correlation coefficients. Functional properties of greatest importance were, as expected, swelling and gel strength. Swelling explained 59% of the variance in a solution with a correlation coefficient of 0.79 and gel strength explained 76% of the variance in a solution with a correlation coefficient of 0.88. The results have shown that well-defined model systems prepared under controlled conditions were useful for the identification and estimation of relationships between functional properties and properties of meat systems. Even if only approximate statistical models were used, the possibilities of functional properties as predictors for the changes in properties of an industrially processed product containing as little as 4% added proteins were strongly indicated.

## CONCLUSIONS

The following general conclusions can be made from this work:

1. It is possible to establish simple methods for functional properties that give information on how a new protein will act in a food system.
2. Properties dependent on water-protein interactions were chosen for this study. They were solubility, viscosity, swelling and gelation. These properties were not independent and the relationship could be statistically calculated by a hierarchical cluster analysis. The relationships could also be rationalized on a physico-chemical basis, in which the quaternary structure and the balance between attractive and repulsive forces played a dominant role for all the properties studied.





3. The functional properties were highly dependent on variables such as pH, salt concentration, protein concentration and temperature. The dependence on these variables can be explained in terms of effect on the balance between repulsive and attractive forces, and thereby on effect on the molecular structure.
4. Model food systems, prepared under controlled conditions were useful for the identification and estimation of relationships between functional properties of added proteins and properties of food systems. Very high correlations were obtained between the functional properties studied and the water-binding properties of model food systems by a statistical regression procedure. Correlation coefficients as high as 0.97 - 0.99 were obtained.
5. When as little as 4% protein was added to a real meat product, differences in functional properties influenced the texture of the finished food product. It was even possible to predict changes in texture properties from statistical models with functional properties as independent variables. As the regression equations used were estimated from changes in moisture loss, these changes and those of texture must be similar in character.
6. The ability of the protein to form a gel structure and imbibe water was shown to be of great importance both for the water-binding and the texture properties of heat-treated meat systems.
7. The properties studied in this work can be used for functional characterization of a new protein product. Furthermore, relationships established strongly indicate the use of functional properties as valuable tools for the optimization of processes for protein production as well as for food production.

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PROJECT DESIGN

